Abstract

β-Carotene, the most abundant provitamin A carotenoid in the diet, is converted to retinal by β-carotene 15,15'-monooxygenase (BCMO1). However, β-carotene absorption and conversion into retinal is extremely variable among individuals, with proportions of low responders to dietary β-carotene as high as 45%. Recently, 2 common nonsynonymous single nucleotide polymorphisms (SNPs) within the BCMO1 coding region (R267S; rs12934922 and A379V; rs7501331) revealed reduced catalytic activity, confirming that genetic variations contribute to the low responder phenotype. Because 4 SNPs 5’ upstream from the BCMO1 gene were recently shown to affect circulating carotenoid concentrations, the current study aimed to investigate the effects of these SNPs on β-carotene conversion efficiency. Three of the 4 polymorphisms (rs6420424, rs11645428, and rs6564851) reduced the catalytic activity of BCMO1 in female volunteers by 59, 51, and 48%, respectively. The TG-rich lipoprotein fraction retinyl palmitate:β-carotene ratio was negatively correlated with the G allele of rs11645428 (r = −0.44; P = 0.018), whereas it was positively correlated with the G allele of rs6420424 (r = 0.53; P = 0.004) and the T allele of rs6564851 (r = 0.41; P = 0.028). Furthermore, large inter-ethnic variations in frequency of affected alleles were detected, with frequencies varying from 43 to 84% (rs6420424), 52 to 100% (rs11645428), and 19 to 67% (rs6564851). In summary, a range of SNPs can influence the effectiveness of using plant-based provitamin A carotenoids to increase vitamin A status in at-risk population groups and this effect may vary depending on ethnic origin. J. Nutr. 142: 1615–1655, 2012.

Introduction

Vitamin A is essential for normal growth and development, immune system, vision, and other functions in the human body. Because humans are unable to synthesize vitamin A de novo, they must consume diets with preformed vitamin A or provitamin A carotenoids. Vitamin A deficiency is a serious public health problem that mostly affects pregnant and lactating women and preschool children, with an estimated 250 million at risk of developing vitamin A deficiency disorders (1). However, low vitamin A intakes have also been described in Western societies. Indeed, a majority of the vitamin A requirements for the U.K. population are not met by dietary intake of...
preformed retinol, and 15% of young individuals aged 19–24 y have a total vitamin A intake below the lower recommended nutrient intake level (2,3).

Upon absorption, provitamin A carotenoids are readily converted to vitamin A by BCMO19 expressed in enterocytes of the intestinal mucosa (4). β-Carotene is the most abundant provitamin A carotenoid in the diet and ~95% of retinolides arising from β-carotene are produced by this pathway in vivo (5). However, β-carotene conversion efficiency during absorption varies widely from person to person, even within studies that were conducted in relatively homogeneous groups (6–10). Indeed, in double-tracer studies, 27–45% of volunteers have been classified as poor responders (8–10).

Genetic variability in β-carotene metabolism may provide an explanation for the molecular basis of the poor responder phenotype within the population. Recently, two nonsynonymous SNP (R267S and A379V) in the human BCMO1 gene were identified with variant allele frequencies of 42 and 24%, respectively (11). Responsiveness to a pharmacological dose of β-carotene in a human intervention study revealed that these SNPs were associated with reduced β-carotene conversion efficiency of 32–69% (11).

In an attempt to identify genetic factors affecting fasting carotenoid concentrations, a genome-wide association study identified four novel common variants associated with circulating carotenoid concentrations in the InCHIANTI study, the Women's Health and Aging Study, and the α-Tocopherol, β-Carotene Cancer Prevention study (12). The four identified SNP (rs6420424, rs8044334, rs11645428, and rs6564851) are based 7.7 kb upstream from the BCMO1 gene. Although the SNP rs6564851 showed the strongest association with plasma β-carotene, the authors did not investigate the effect of these four SNP on provitamin A conversion efficiency.

The aim of this study was to investigate the effects of the four identified SNP 5′ upstream from the BCMO1 gene on β-carotene conversion efficiency. We found that three of the four intronic SNPs reduced the catalytic activity of BCMO1 in female volunteers and that the affected alleles showed a large frequency range between different ethnic groups.

**Materials and Methods**

**β-Carotene supplementation study.** Twenty-eight female volunteers from the UK took part in the β-carotene supplementation study as described by Leung et al. (11). Informed and written consent was obtained from all volunteers and ethical permission was obtained from the Newcastle and North Tyneside Local Research Ethics Committee. Volunteers fasted for 12 h prior to consuming a single oral dose of 120 mg β-carotene (10% SWS from DSM Nutritional Products) together with a fat-rich meal. The test meal was designed to reflect the same nutrient content as described by Borel et al. (6) and consisted of a muffin, fruit yoghurt, and water. Fasting blood samples (20 mL) were collected on the morning of the experiment and a second blood sample (20 mL) was taken 3 h after consumption of the test meal. During the 3 h, volunteers were allowed to drink only water. Plasma and TRL preparation as well as HPLC analysis of these samples were performed as described by Leung et al. (11). In brief, plasma samples were obtained from 10 EDTA Vacutainers (Southern Syringe Service) after centrifugation at 1500 × g for 10 min at 4°C. Subsequently, TRL samples were obtained after plasma centrifugation at 435,680 × g for 1 h 38 min at 4°C in an Optima Max ultracentrifuge (Beckman Coulter). Using a Beckman CentriTube, slicer tubes (Beckman Coulter) were sliced and 0.3 mL of the top layer was removed and the lid was washed with 0.35 mL of NaCl solution to remove any residues. Samples were covered in foil to prevent oxidation of β-carotene by sunlight. A total of 100 µL of plasma or 600 µL of TRL was deproteinized with 1 mL of analytical-grade ethanol (VWR) containing 10 µL of trans β-apo-8-carotene as the internal standard and extracted with 2 mL of analytical-grade hexane containing 0.01% BHT and vortex mixed. After centrifugation at 1300 × g and 4°C for 15 min, the hexane layer was removed and evaporated under a stream of nitrogen. Dried residues were redissolved in 200 µL of HPLC grade acetonitrile/chloroform/methanol (70:15:10 v/v/v) (Fisher Scientific) and 40 µL/sample was injected for analysis of retinyl esters and carotenones using a Dionex HPLC system as described by Leung et al. (11). Nutrient analysis was performed using Windiets 2005 as previously described (11).

Genotyping DNA samples for rs12934922, rs7501331, rs6420424, rs8044334, rs11645428, and rs6564851. SNP analysis was performed on the MassARRAY system (Sequenom) applying the iPLEX method and MALDI-TOF MS for analyte detection. All reactions were performed according to the standard protocol recommended by the system supplier. In brief, the iPLEX process relies on a small-volume PCR amplifying the target regions, including the SNP position, in a multiplex fashion. After PCR, the unconsumed dNTP were removed by treatment with shrimp alkaline phosphatase. During the following iPLEX reaction, a MassEXTEND primer, which anneals directly adjacent to the SNP position, was elongated to generate the allele-specific analytical products. Details of all primers are given in Supplemental Table 1. The products, which differed by mass according to the incorporated bases, were then introduced into the mass spectrometer (MassARRAY Analyzer Compact) and data were fully automatically acquired and analyzed in a real-time setting. Data analysis was performed with the TYPER RT software version 3.4. A Tagging close proximity of the analyzed SNPs, rs6420424 could not be run in parallel with rs12934922, rs7501331, rs8044334, rs11645428, and rs6564851. Therefore, we designed the assay with rs12597639 as a tag SNP for rs6420424 with an LOD of 26.1 (\( r^2 = 0.87 \)), allowing the analysis of all six SNPs in one assay.

**Statistical analysis.** Statistical analyses were performed by using the SPSS software package version 17.0. Results are expressed as means ± SEM. Plasma β-carotene, TRL retinyl palmitate, and TRL β-carotene were ln-transformed to improve normality. Pearson correlation analysis was performed on ln-transformed fasting β-carotene and TRL retinol palmitate:β-carotene ratios. A multivariate General Linear Model analysis was carried out using fasting plasma β-carotene and TRL retinol palmitate:β-carotene ratio as the dependent variables and age, BMI, dietary intake of β-carotene, and preformed retinol as the covariates. Pairwise comparisons were adjusted for multiple comparisons by Bonferroni. A level of P < 0.05 was accepted as significant.

**Results**

Twenty-eight female volunteers (mean age = 20 y, mean BMI = 22 kg/m²) took part in the study. The mean daily total energy intake was 6.4 MJ, with carbohydrates, protein, fat, and alcohol contributing 50.4, 18.6, 28.5, and 2.4% of total energy intake, respectively. The mean daily total provitamin A carotene intake was 2.5 mg, with β-carotene being the main ingested provitamin A carotene (mean β-carotene concentration = 1.2 mg, range = 0.2–7.0 mg/d). The mean daily intake of preformed retinol was low (133 µg/d). A total of 36% of women had retinol equivalent intakes above the recommended nutrient intake of 600 µg/d and 14% had intakes below the lower recommended nutrient intake of 250 µg/d. Fasting mean plasma concentrations for β-carotene and retinol were 0.36 µmol/L (95% CI = 0.29–0.45 µmol/L) and 1.52 µmol/L (95% CI = 1.36–1.69 µmol/L), respectively. All volunteers had adequate serum vitamin A concentrations > 1.05 µmol/L. Age, BMI, plasma TG concentrations, and dietary
vitamin A and β-carotene intake were not related to fasting plasma β-carotene and retinol concentrations.

Genotype frequencies were determined for rs6420424, rs8044334, rs11645428, and rs6664851 in the current study (Table 1). Allele frequencies for the A and G allele of rs6420424 were 45 and 55%, for the G and T allele of rs8044334 were 30 and 70%, for the A and G allele of rs11645428 were 29 and 71%, and for the G and T allele of rs6664851 were 48 and 52%, respectively.

SNP frequencies for 11 different ethnic population groups obtained from the HapMap database (13) are displayed for rs6420424, rs8044334, rs11645428, and rs6664851 (Table 2).

The current study indicated that fasting plasma β-carotene concentration was positively correlated with the G allele of rs11645428 (r = 0.44; P = 0.02), whereas it was negatively correlated with the T allele of rs6664851 (r = −0.38; P = 0.048). After adjustment for age, BMI, and dietary vitamin A and β-carotene intakes, general linear model analysis showed that homozygous carriers of the T allele of rs6664851 had lower fasting plasma β-carotene concentrations of 317.1 nmol/L (P = 0.024) compared to 697.2 nmol/L for homozygous G allele carriers of rs6664851 (Fig. 1). Homozygous carriers of the G allele of rs11645428 tended to have higher fasting plasma β-carotene concentrations of 542.3 nmol/L (P = 0.065) compared to 242.8 nmol/L for homozygous A allele carriers of rs11645428 (Fig. 1).

To test if the rs6420424, rs8044334, rs11645428, and rs6664851 variant alleles could influence the ability of an individual to convert β-carotene into retinol, retinyl palmitate and β-carotene concentrations were measured in the TRL 3 h after a single 120-mg dose of β-carotene. All 28 volunteers showed a β-carotene and retinyl palmitate response measured in the TRL fraction as previously described (11). The retinyl palmitate:β-carotene ratio was used to determine β-carotene conversion efficiency in each participant. The TRL retinyl palmitate:β-carotene ratio was negatively correlated with the G allele of rs6420424 (r = 0.53; P = 0.004) and the T allele of rs6664851 (r = 0.41; P = 0.028).

General linear model analysis showed that after adjustment for age, BMI, dietary retinol, and β-carotene intake, homozygous G allele carriers for rs11645428 had lower TRL retinyl palmitate:β-carotene ratios of 0.33 (P = 0.004) compared to 0.67 observed in A allele carriers (Fig. 1). Additionally, homozygous G allele carriers for rs6420424 and homozygous T allele carriers for rs6664851 had higher TRL retinyl palmitate:β-carotene ratios of 0.60 (P = 0.002) and 0.63 (P = 0.003), respectively, compared to the observed ratios of 0.24 and 0.33 in the other alleles (Fig. 1). Interestingly, an interaction between rs6420424 and rs11645428 on TRL retinyl palmitate:β-carotene ratios in A379V C allele carriers was observed (Fig. 2). In this subgroup analysis, carriers of the reference alleles of rs6420424 and rs11645428 affected β-carotene conversion efficiency in opposite directions. The result highlights the importance of genotyping both exonic and intronic BCMO1 SNPs to investigate β-carotene conversion efficiency in different population groups due to their reported differing frequencies (Table 2).

**TABLE 2** Allele frequencies of rs11645428, rs6420424, rs8044334, and rs6664851 in 11 different ethnic groups

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<th>CHB</th>
<th>CHD</th>
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<td>41.4</td>
<td>19.2</td>
<td>46.1</td>
<td>11.6</td>
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</table>

1 According to Hapmap data (13). ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California; MKK, Maasai in Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria.

**TABLE 1** rs6420424, rs8044334, rs11645428, and rs6664851 SNP frequencies in 28 female volunteers from the UK

<table>
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<tr>
<th>Genotype</th>
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<th>rs11645428</th>
<th>rs6664851</th>
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<td>6 (21.4)</td>
<td>8 (28.6)</td>
</tr>
<tr>
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<td>13 (46.4)</td>
<td>4 (14.3)</td>
<td>11 (39.3)</td>
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<tr>
<td>G</td>
<td>11 (39.3)</td>
<td>13 (46.4)</td>
<td>18 (64.3)</td>
<td>9 (32.1)</td>
</tr>
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</table>

1 SNP, single nucleotide polymorphism.
2 Analyzed using rs12597639 as the tag SNP.

**Discussion**

Previous work indicated that two common nonsynonymous SNPs in the *BCMO1* gene, occurring at frequencies similar to those of the poor responder trait, reduced catalytic activity of *BCMO1* by up to 69% in female volunteers (11). To our knowledge, the current work shows for the first time that common SNP upstream of *BCMO1* also reduce the conversion of β-carotene into retinoids by 48 to 59%. More importantly, SNPs negatively affecting provitamin A conversion efficiency occurred at high allele frequencies between 30 and 71%. The observed variable response to β-carotene, which has led to the characterization of the poor responder phenotype in up to 45% of volunteers in double-tracer studies (8–10), is therefore most likely caused by a combination of nonsynonymous and intronic SNPs within and upstream of *BCMO1*.

Interestingly, carriers of the reference alleles of rs6420424, rs11645428, and rs6664851 showed an increase in the conversion efficiency, whereas carriers of the reference allele of rs11645428 indicated reduced retinyl-palmitate:β-carotene ratios (Fig. 1). More importantly, because the reference alleles of rs6420424 and rs11645428 affect β-carotene conversion efficiency in opposite directions, the current study indicates a SNP/SNP interaction on TRL retinyl palmitate:β-carotene ratios with SNPs influencing provitamin A conversion.
consequences for volunteers who are A379V C allele carriers (Fig. 2). A379V C allele carriers have previously been identified as the most efficient provitamin A converters (11). rs11645428 is particularly interesting, because the majority of the genotyped human population within HapMap has the homozygous G allele (Table 2). Homozygous rs11645428 G allele carriers are associated with a 51% decrease in conversion efficiency compared to homozygous A allele carriers (Fig. 1). Likewise, homozygous A allele carriers of rs6420424 show a reduced conversion efficiency by 59% compared to the homozygous G allele carriers. The homozygous A allele of rs6420424 occurs at a frequency of ~70% in Asian populations yet at only ~30% in European and American or at ~20% in African ethnic populations (Fig. 1; Table 2). Given that the frequencies of both the G allele (rs11645428) and A allele (rs6420424) are significantly higher in Asian compared to European ethnic groups (Table 2), one could speculate that observed conversion efficiency in A379V C allele carriers would be lower in Asian compared to European ethnic groups if both rs6420424 and rs11645428 are not detected. This observation, together with the fact that allele frequencies for rs7501331 have been found to be widely different between ethnic groups (11), indicates the importance of factoring in genetic variation and ethnic origin when provitamin A activities of plant-based foods are determined.

Baseline retinol and \(\beta\)-carotene concentrations in the current study were similar to previous studies, with values ranging from 1.4 to 1.7 \(\mu\)mol/L and 288 to 453 nmol/L, respectively (6,9,14). The SNPs rs6420424, rs8044334, rs11645428, and rs6564851 were very strongly associated with fasting \(\beta\)-carotene concentrations in 3 independent studies with \(P\) values \(<1 \times 10^{-12}\) as determined by meta-analysis (12). In accordance with the genome-wide association study, we also observed that rs6564851 had the strongest association with fasting \(\beta\)-carotene concentrations.

We used a pharmacological dose of 120 mg/d \(\beta\)-carotene in the current study. This was done to overcome the problem of postprandial release of \(\beta\)-carotene from a previous meal into the TRL fraction. Studies by Borel et al. (6) and van Vliet et al. (15) have shown that absorbed \(\beta\)-carotene is not entirely released after the first postprandial period but can be stored in intestinal enterocytes and incorporated into chylomicrons after subsequent meals containing no \(\beta\)-carotene. Although participants...
were instructed to fast for 12 h prior to the current intervention, it is possible that the high-fat meal they consumed cleared out both β-carotene and retinyl-palmitate present in the intestine from a previous meal. Consequently, this would affect the reliability of the retinyl-palmitate:β-carotene ratio if physiological concentrations of β-carotene were administered, especially because diets prior to the current intervention were not standardized. Although a pharmacological dose of ingested β-carotene significantly reduces bioefficacy (16), it also allows the distinction to be clearly made between β-carotene nonresponders and responders (6,17). Borel et al. (6) reported no incidences of β-carotene nonresponders after ingestion of 120 mg β-carotene but still observed participants who were β-carotene low responders. Although the current investigation applied the same pharmacological dose as the study by Borel et al. (6), a 4.5-fold higher TRL β-carotene and a 2.2-fold higher TRL retinyl palmitate response were observed in the present study compared to values reported by Borel et al. (6). The major difference between this study and the study by Borel et al. (6) was the formulation of administered β-carotene; a water-soluble beadlet form was given in the current study, whereas Borel et al. (6) gave β-carotene in an oil suspension. Indeed, the current study provides evidence that absorption as well as conversion is higher when β-carotene is given in the form of water-soluble beadlets, even if the relative conversion efficiency is reduced from 1.0 (6) to 0.38 (current study). Furthermore, administration of β-carotene as water-soluble beadlets produced a decrease in the CV of TRL retinyl palmitate:β-carotene from 221% reported by Borel et al. (6) to a CV of 60% in the current study. Although the total vitamin A status in participating volunteers was not measured, the current study was clearly able to indentify that common SNPs upstream and within the BCMO1 gene influence the ability to cleave provitamin A carotenoids even in a situation where the intestinal cell is saturated with a pharmacological dose of β-carotene.

In conclusion, the present study shows that three common intronic SNPs upstream of the human BCMO1 gene alter β-carotene metabolism similar to previously identified non-synonymous SNPs within the gene. Female volunteers carrying the A allele (rs6420424), G allele (rs11645428), and G allele (rs6564851) of these intronic SNPs showed a reduction in conversion efficiency by up to 59% combined with higher fasting β-carotene concentrations. Thus, this study further demonstrates that provitamin A metabolism is influenced by multiple SNPs and that genetic variability should be taken into account in future recommendations for provitamin A supplements. The importance for local recommendations and policies is further strengthened by the observation of clear differences in SNP frequencies across different ethnic groups.

Acknowledgments

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Literature Cited